Pestic. Sci. 1998, **53**, 285–290

Carbofuran-Induced Biochemical Changes in *Clarias batrachus*

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(Received 30 September 1997; revised version received 24 February 1998; accepted 10 March 1998)

Abstract: The effect of carbofuran, an organo-carbamate pesticide, upon the level of protein as well as the activity of lactate dehydrogenase (EC 1.1.1.28, LDH) was studied by exposing the teleost fish, *Clarias batrachus*, to different subacute concentrations (0·01 and 0·02 mg litre^{-1}) for 96 h and 15 days. The results showed a drastic decrease in the protein content in different body organs of the fish. The pesticide also caused a significant decrease in the level of activity of LDH in different body tissues of the fish, the effect being more pronounced in the gills, muscle, brain and liver than in kidney and heart. The decrease in protein content and the activity of LDH in fish tissues was more marked at the higher concentration of the pesticide for the longer duration of treatment. The results suggested that carbofuran has an effect at very low concentration (compared to its LC_{50} value) possibly at the level of protein metabolism, and also inhibits the activity of LDH, the terminal glycolytic enzyme. © 1998 SCI

Pestic. Sci., **53**, 285–290 (1998)

Key words: carbofuran; protein; lactate dehydrogenase; inhibition; glycolytic enzyme

1 INTRODUCTION

Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) is known to produce manifestations of hypercholinergic activity involving central as well as peripheral organs[1,2] by inhibiting acetylcholinesterase at synapses in the brain and neuromuscular junctions.[3,4] Lactate dehydrogenase (EC 1.1.1.28, LDH), a cytoplasmic biomarker in the glycolytic pathway

$$\text{pyruvate} + \text{NADH} + \text{H}^+ \overset{\text{LDH}}{\rightleftharpoons} \text{lactate} + \text{NAD}^+$$

plays an important role in regulating glycolysis and is therefore crucial for normal cellular functions. The activity of LDH is present in virtually all tissues of the organism. In most cases of tissue damage, whether from a disease process or a toxic compound, the activity of LDH is reported to be significantly affected.[5] The degree of alteration in the activity of such a cellular enzyme depends primarily on the magnitude and severity of cell damage.

The effects of different groups of pesticides, mainly the organochlorines and organophosphates, on the biochemical parameters in several aquatic organisms, including fish, have already been studied and the toxicity of these compounds at very low concentration has been established. Because of their generally high lipid solubility, coupled in some cases with poor biodegradability and high toxicity, organochlorines have been replaced by other classes of pesticide, which have high insecticidal efficacy and relatively fast biodegradability.[6] The information available regarding the biochemical behaviour of the pesticides of the carbamate group, particularly carbofuran, using aquatic organisms as experimental models is scanty. The present communication describes an investigation of the effect of carbofuran on the non-target aquatic teleost, *Clarias batrachus*, by exposing it to sublethal concentrations of the pesticide for varying treatment durations.

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Contract/grant sponsor: All India Council for Technical Education.
Contract/grant number: 802-1/RD 11/R&D/94 Rec 246.

 Pestic. Sci. 0031-613X/98/$17.50. Printed in Great Britain

2 EXPERIMENTAL METHODS

2.1 Fish

Healthy specimens of the freshwater teleost, *C. batrachus* (length 15–20 cm, body weight, 70–90 g) were obtained from a local fish market and brought to the laboratory in 20-litre-plastic containers. The fish were checked for external signs of injury or disease, any such fish being discarded and only healthy fish used for experimentation. Fish were acclimatized to laboratory conditions under natural photoperiod and ambient temperature for at least seven days in glass aquaria containing tap water, which was replaced with fresh water every 24 h. The physicochemical characteristics of water were analysed according to known procedures.[7] The fish were fed flour pellets/egg albumin daily after the aquaria were cleaned. However, fish were not fed 12 h prior to their transfer into the experimental jars for the sub-acute toxicity tests.

2.2 Bioassay

Carbofuran (technical grade 99·63% purity, acetone-soluble) was purchased from Rallis India Limited and its LC_{50} value was determined using standard procedures.[7] The stock solution of active ingredients of the pesticide was prepared by dissolving the compound in acetone. The fish were exposed to subacute concentrations of carbofuran (0·01, 0·02 mg litre^{-1}) for two different incubation periods of 96 h and 15 days. Controls were maintained in a pesticide-free medium. Acetone alone was added to the control aquaria. After the stipulated periods, the fish were dissected and the tissues (liver, gills, muscle, brain, heart and kidney) were isolated in ice-cold condition for further study).

2.3 Preparation of cell-free extracts

The tissues (liver, gills, muscle, brain, heart and kidney) were thoroughly washed in normal saline and homogenized (10%, w/v) in 0·25 M sucrose solution for one minute using a Potter–Elvehjam homogenizer with a Teflon-coated pestle under ice-cold condition. The homogenates were kept for 30 min in cold with intermittent stirring and centrifuged at 10 000*g* for 30 min in a refrigerated high-speed centrifuge. The supernatant of each tissue homogenate was collected and kept in an ice bath for 10 min and used as enzyme source.

2.4 Assay of lactate dehydrogenase (LDH)

LDH activity in the cell-free extracts was measured by a NADH-linked optical assay following the methods of Horecker and Kornberg.[8] The reaction mixture (3 ml), in quartz cuvettes of 1-cm light path, contained NADH (2·4 mM), sodium pyruvate (50 mM), Tris-HCl buffer (0·2 M, pH 7·4), KCl (0·1 M) and cell-free extracts (10–20 µl, equivalent to 50–100 µg protein). The reaction was started by adding the enzyme source and decrease in extinction at 340 nm was read at 30-s intervals to monitor the rate of oxidation of NADH to NAD^+ (a measure of reduction of pyruvate to lactate) on a Digispec-200 GL UV-Vis. spectrophotometer. One unit (U) of activity of LDH was expressed in terms of µmoles sodium pyruvate reduced ml^{-1} min^{-1}. The estimation of protein in different cell-free extracts was done by the known method.[9] The cell-free extracts were mixed separately with an equal volume of TCA (100 g litre^{-1}), kept for 5 min at 10°C and centrifuged at 800*g* for 20 min at 4°C. The precipitates were dissolved in a suitable volume of NaOH (0·1 M) and aliquots from these solutions were processed for the determination of protein. Bovine serum albumin (BSA) was used as standard protein.

3 RESULTS

Carbofuran induced significant change in the protein content and the activity of lactate dehydrogenase in certain tissues of *C. batrachus*, exposed to sublethal concentrations (0·01 and 0·02 mg litre^{-1}) for different periods (96 h and 15 days). The results, presented in Tables 1 and 2, showed that the average protein contents in various tissues of *C. batrachus* differed significantly from those of the control. The average protein concentrations in the tissues of control fish were in the following order: liver > heart > brain > muscle > gills > kidney, the values being 42·13 (±0·22), 27·49 (±0·26), 22·99 (±0·12), 22·64 (±0·18), 20·52 (±0·56), 19·82 (±0·23) mg g^{-1} wet weight of liver, heart, brain, muscle, gills and kidney respectively. The level of protein decreased in the tissues of *C. batrachus* due to exposure to carbofuran at both subacute concentrations (0·01 and 0·02 mg litre^{-1}) and treatment durations (96 h and 15 days). When the fish were treated with carbofuran (0·01, 0·02 mg litre^{-1}) for a 96-h exposure period, the protein level decreased more sharply in the fish brain and muscle than in other tissues. The liver and kidney, however, showed similar trends of decrease in protein level at both concentrations of carbofuran, whereas the gills were least affected. The protein level was found to be lowered in all the tissues at a high concentration of the pesticide (0·02 mg litre^{-1}). In this case the order of decrease in protein content in the fish tissues was different from the trend obtained when fish was exposed to a lower concentration of carbofuran and was as follows; brain > muscle > liver > heart > kidney > gills. The results are shown in Table 1.

The level of protein in different fish tissues was further decreased when the fish were treated with carbo-

TABLE 1
Effect of Carbofuran on the Protein Content of Different Tissues of *Clarias batrachus*, Exposed for 96 h

	Protein content (mg g^{-1} wet weight of tissue) ($\pm SEM$)[abc]		
	Carbofuran (mg $litre^{-1}$)		
Tissue	*0*	*0·01*	*0·02*
Liver	42·13 (±0·22)	34·26 (±0·31)*** [−18·68]	30·00 (±0·52)*** [−28·79]
Heart	27·49 (±0·26)	24·03 (±0·24)*** [−12·59]	22·50 (±0·31)*** [−18·15]
Brain	22·99 (±0·12)	16·50 (±0·28)*** [−28·23]	15·06 (±0·58)*** [−34·49]
Muscle	22·64 (±0·18)	16·59 (±0·25)*** [−26·77]	15·61 (±0·71)*** [−33·03]
Gills	20·52 (±0·56)	18·96 (±0·17) [−7·602]	18·03 (±0·79)* [−12·13]
Kidney	19·82 (±0·23)	16·21 (±0·22)*** [−18·21]	15·36 (±0·27)*** [−22·50]

[a] $n = 3$.
[b] Values in brackets are percentage change from control.
[c] Significantly different from control at $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$ (Students *t*-test).

furan for a longer incubation period (15 days). The results shown in Table 2 demonstrated that the protein levels in fish brain, kidney and muscle decreased by 44, 45 and 49% respectively at the higher carbofuran concentration (0·02 mg $litre^{-1}$), as compared to control. The decrease in protein content at both concentrations of the pesticide was in the order muscle > kidney > brain > liver > heart > gills. The data presented in Tables 1 and 2 indicate that the carbofuran was drastically affecting the brain of *C. batrachus* after a short period (96 h) of pesticide treatment, whereas after a longer treatment period (15 days), the pesticide caused

TABLE 2
Effect of Carbofuran on the Protein Content of Different Tissues of *Clarias batrachus*, Exposed for 15 Days

	Protein content (mg g^{-1} wet weight of tissue) ($\pm SEM$)[abc]		
	Carbofuran (mg $litre^{-1}$)		
Tissue	*0*	*0·01*	*0·02*
Liver	40·42 (±0·46)	28·97 (±0·35)*** [−28·32]	25·12 (±0·31)*** [−37·85]
Heart	25·30 (0·26)	19·74 (±0·40)*** [−21·97]	18·22 (±0·49)*** [−27·98]
Brain	22·46 (±0·37)	14·30 (±0·37)*** [−36·33]	12·56 (±0·64)*** [−44·07]
Muscle	22·13 (±0·37)	13·21 (±0·26)*** [−40·30]	11·32 (±0·31)*** [−48·84]
Gills	19·10 (±0·34)	15·80 (±0·35)*** [−17·27]	14·10 (±0·37)*** [−26·17]
Kidney	20·92 (±0·37)	14·11 (±0·28)*** [−32·55]	11·60 (±0·59)*** [−44·55]

[a] $n = 3$.
[b] Values in brackets are percentage change from control.
[c] Significant difference from control at $*P < 0{\cdot}05$, $**P < 0{\cdot}01$; $***P < 0{\cdot}001$ (Students *t*-test).

TABLE 3
Effect of Carbofuran on the Activity of Lactate Dehydrogenase from *Clarias batrachus*, Exposed for 96 h

	LDH activity ($U\ g^{-1}$ *wet weight*) ($\pm SEM$)[abc]		
	Carbofuran (*mg litre*$^{-1}$)		
Tissue	*0*	*0·01*	*0·02*
Liver	59·90 (±1·05)	55·92 (±1·36)* [−6·65]	52·40 (±1·44)* [−12·52]
Heart	54·07 (±0·81)	51·04 (±2·3) [−5·61]	46·67 (±1·46)* [−13·68]
Brain	51·00 (±1·37)	48·43 (±1·45) [−5·03]	44·30 (±1·43)* [−13·13]
Muscle	44·12 (±1·23)	41·60 (±2·18) [−5·71]	38·15 (±1·29)* [−13·53]
Gills	39·89 (±1·43)	36·00 (±1·73) [−9·75]	33·72 (±1·477)* [−15·46]
Kidney	32·52 (±0·94)	30·11 (±1·18) [−7·41]	27·64 (±1·06)* [−15·00]

[a] $n = 3$.
[b] Values in brackets are percentage change from control.
[c] Significant difference from control at $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$ (Students *t*-test).

sharp decreases in the protein contents of muscle, kidney and brain, followed by liver, heart and gills. The results demonstrated that carbofuran at low concentration (0·01 mg litre^{-1}) and short exposure period (96 h) was exerting less toxic effect than that at the higher concentration (0·02 mg litre^{-1}) for a longer treatment duration (15 days).

The effect of carbofuran on the activity of LDH in different body organs of *C. batrachus* exposed to two sublethal concentrations of carbofuran (0·01 and 0·02 mg litre^{-1}) for 96 h and 15 days treatment periods was monitored. The results are shown in Tables 3 and 4. The enzyme activity was found to be highest in liver and lowest in kidney of the control fish. The tissues

TABLE 4
Effect of Carbofuran on the Activity of Lactate Dehydrogenase from *Clarias batrachus*, Exposed for 15 Days

	LDH activity ($U\ g^{-1}$ *wet weight*) ($\pm SEM$)[abc]		
	Carbofuran (*mg litre*$^{-1}$)		
Tissue	*0*	*0·01*	*0·02*
Liver	58·76 (±1·73)	46·33 (±1·23)** [−21·15]	39·48 (±1·41)*** [−32·81]
Heart	54·35 (±1·81)	41·14 (±1·46)*** [−24·30]	35·69 (±1·75)** [−34·33]
Brain	50·36 (±1·02)	40·23 (±1·15)** [−20·11]	33·75 (+1·23)** [−33·78]
Muscle	44·67 (±1·34)	34·16 (±1·17)* [−23·52]	29·43 (±1·44)*** [−34·11]
Gills	40·02 (±1·21)	25·34 (±1·84)** [−36·68]	22·75 (±1·14)*** [−43·15]
Kidney	31·93 (±0·96)	25·1 (±0·51)** [−21·39]	22·69 (±0·65)** [−28·93]

[a] $n = 3$.
[b] Values in brackets are percentage change from control.
[c] Significant difference from control at $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$ (Students *t*-test).

exhibited the following order of LDH activity: liver > muscle > heart > brain > gills and kidney; the values being 59·9 (±1·05), 54·07 (±0·81), 51·0 (±1·37), 44·12 (±1·23), 39·89 (±1·43) and 32·52 (±0·94) U g^{-1} wet weight of tissue respectively. The exposure of *C. batrachus* to carbofuran resulted in substantial reduction in the LDH activity. The results indicated that carbofuran at sublethal concentrations exerted an inhibitory effect on LDH activity after both periods of treatment. The decrease in enzyme activity was more pronounced when the fish was exposed to the pesticide at the higher concentration for the longer period of exposure (15 days) than that at the lower concentration for the shorter treatment duration (96 h). Carbofuran caused maximum inhibition of LDH activity in gills, whereas smaller changes were recorded in other tissues of *C. batrachus* (Tables 3 and 4).

4 DISCUSSION

The results obtained in the present study demonstrated that carbofuran induced changes in the levels of protein content as well as LDH activity in the different tissues of *C. batrachus* exposed to sublethal concentrations of pesticide (0·01 and 0·02 mg $litre^{-1}$) for incubation periods of 96 h and 15 days. The data presented in Tables 1 to 4 indicate that the carbofuran significantly decreased the protein content as well as the activity of LDH at low concentrations (about one-tenth of the LC_{50} value) suggesting thereby toxicity of carbofuran to the fish. The results showing the influence of the pesticide on the level of protein may be attributed to enhanced activities of transaminases (GOT and GPT) in fish tissues (Singh, R. K. & Sharma, B, unpublished results). However, similar observations have been reported in the freshwater murrel, *C. punctatus*, exposed to subacute concentrations of malathion and carbofuran.[10] At sublethal concentrations, pesticides have been shown to induce significant changes in the energy metabolism,[11] neurotransmission,[12] immune system,[6] and the activity of certain key enzymes[13–15] in the freshwater fish. The LDH activity in tissues of carbofuran-exposed *C. batrachus* decreased significantly at both concentrations of pesticide tested and both durations of incubation. The decrease in the activity of LDH from different organs of *C. batrachus* demonstrated a linear correlation with increase in the carbofuran concentration and period of treatment. The results are in agreement with the observations reported in freshwater crab exposed to methyl parathion,[16] in rat treated with carbofuran[14] and in *C. batrachus* exposed to sublethal concentrations of carbaryl.[15] The results of *in-vitro* studies conducted to determine the effect of carbofuran showed that the pesticide strongly inhibited the activity of LDH isolated from the tissues of *C. batrachus* (Singh, R. K. & Sharma, B. unpublished data). The finding suggested that the inhibitory effect of organocarbamate pesticides on this terminal glycolytic enzyme may be due to the formation of enzyme-inhibitor complex, thereby reducing the activity of LDH leading to impairment of carbohydrate metabolism in *C. batrachus*. Earlier reports from this laboratory[15] have shown that carbaryl (an organocarbamate) at subacute concentrations caused drastic elevation in the level of lactic acid in fish tissues, which was attributed to damage to the kidney, which was not able to flush out excess lactate from the tissues in order to establish homeostasis. Possibly the inhibition in LDH activity and rise in lactic acid levels in the tissues of pesticide-treated *C. batrachus* indicate that the rate of glycolysis in tissues was higher and that of TCA cycle was lower in the teleost. It has also been reported that carbaryl increased the level of biogenic amines in different parts of the fish brain,[4] and markedly inhibited the activity of acetylcholinesterase in fish tissues,[13] showing carbaryl's neurotoxic effect on *C. batrachus*. The accumulation of lactic acid (data not shown) and inhibition of LDH activity in fish tissues exposed to carbofuran (present investigation) now suggest that the pesticide also interferes with energy metabolism.

In summary, carbofuran exerted deleterious effects in *C. batrachus* at low concentration by causing significant changes in the activity of LDH as well as protein content in different fish tissues. The inhibition of enzyme activity was more prominent in gills, muscle, brain and liver than that in kidney and heart. The toxicity of carbofuran in *C. batrachus* was reflected in a concentration and duration-dependent manner. The results of the present investigation may be useful for assessing early warning signs of pesticide poisoning and an indicator for water quality control.

ACKNOWLEDGEMENT

This work was financially supported by the research grant (no. 802-1/RD II/R&D/94 Rec 246, dated 24.03.1995) from All India Council for Technical Education (AICTE)-New Delhi.

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